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LONG-DISTANCE PHYSICAL CONNECTIONS BETWEEN CHONDROCYTES; CELL-TO-CELL COMMUNICATION WITHIN HYALINE CARTILAGE

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Purpose: Even chondrocytes are metabolically active cells, responsible of the maintenance of the hyaline cartilage during the whole adult life, it is highly accepted in the field that chondrocytes in cartilage do not connect each other, as they are isolated inside their lacunae separated from each other by a distance between 5 to 60μm. In the same lacuna can co-exit several chondocytes interacting between them. However, how the chondrocytes from different lacuna interact between each other and timely respond to physical or chemicals stimuli, are largely unknown. Thus far, the unique communication between chondrocytes in the superficial layer with chondrocytes in the middle and deeper layers it is accepted that only occurs through diffusion. Nevertheless, the intercellular communication confers to a tissue the ability to respond uniformly to localized stimuli. The purpose of this study was to investigate how chondrocytes communicate each other within the matrix and find for some alteration that could explain the degeneration of the matrix that occurs in patients with OA.
Methods: In situ cartilage from human and Sus scrofa were fixed and frozen immediately using Tissue-Tek O.C.T. and isopentanol in liquid nitrogen. Samples were fixed with acetone for confocal optical microscopy assays. Samples were embedded in cacodylate buffer before dehydratation for Scanning Electron Microscope (SEM). Gold was use for coating. Frozen samples were also used for immunofluorescence and immunohistochemistry assays. For total RNA isolation, the proteins and DNA were removed from cell extracts using TRIZOL® Reagent (Invitrogene) before using the RNeasy Kit (Qiagen) which includes DNase treatment (RNAse-Free DNAse Set). Quantifications were done using SYBr green real-time PCR. Virgin Valiunas performed dual voltage-clamp method and whole-cell/perforated patch using chondrocytes from healthy donors and patients with OA.
Results: By using confocal optical microscopy and Scanning Electron Microscopy (SEM), we have found that chondrocytes are physical connected between each other across the extracellular matrix through large cytoplasmic projections. The projections are between 5 to 150 μm in length in human healthy cartilage and young cartilage from Sus scrofa. However condrocytes projections found in OA cartilage are between 10 to 200 μm in length. High levels of expression for the gap junction protein connexin 43 have been detected by RT-PCR and immunohistochemical experiments. We have also found expression of Cx32 and Cx45, however electrophysiology experiments demonstrated that healthy and OA chondrocytes show voltage-dependent behaviour for channels mainly formed by Cx43. We have performed patch clamp experiments to study the interchange of Lucifer Yellow and small molecules of RNA. Our results demonstrated that healthy and OA chondrocytes are able to interchange Lucifer Yellow and small molecules of RNA through Cx43 channels. In fact, our preliminary results showed that inhibition of channels affect the synthesis of collagen type II, MMP3 and MMP9 suggesting that the activity of channels are actively involved with the maintenance of the cartilage matrix.
Conclusions: We have found cell/cell physical connections through all layers of cartilage between chondrocytes located at different distance within cartilage. Intercellular communication occurs through channels voltage-dependent formed by Cx43. Cytoplasmic projections from OA chondrocytes are longer than from healthy chondrocytes, therefore the channel activity can be affected by the electrical depolarization of the membrane in OA chondrocytes. We have found that chondrocytes interchange small RNAs and inhibition of channels affect the expression of matrix-related genes.

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SYNERGISTIC INDUCTION OF ELF3. IN VITRO EFFECT OF LEPTIN AND IL-1 IN HUMAN CHONDROCYTES

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Purpose: To investigate whether leptin, alone or in combination with IL-1β, is able to induce the expression of the novel Ets transcription factor ELF3, and to dissect the role of leptin receptor signalling pathway analyzing the role of several kinases and NF-κB. For completeness, pharmacological modulation by dexamethasone was also investigated.
Methods: The human juvenile costal chondrocyte cell line T/C-28a2 was analyzed for the expression of ELF3 mRNA in resting condition and upon stimulation with leptin alone or in combination with IL-1β by using quantitative RT-PCR. Pharmacological modulation of ELF3 mRNA expression by dexamethasone and the effect of kinases inhibitors such as tyrphostin, wortmannin, LY294002 and SB203580 were also analyzed by qRT-PCR. Ablation of leptin receptor was obtained by using siRNA. The wild type ELF3 promoter and the ELF3 promoter mutated in the NF-κB site were cloned into a luciferase reporter vector and analyzed in transient transfections.
Results: When T/C-28a2 cells were treated with leptin in combination with IL-1β a synergistic induction of ELF3 mRNA expression was observed in comparison to IL-1β treatment alone. Leptin synergism is completely blunted by dexamethasone, by leptin receptor ablation and by selective inhibitors of JAK2 kinase, PI3 kinase and p38 kinase. Leptin synergism is dependent on the integrity of NF-κB binding site in the ELF3 promoter.
Conclusions: Leptin synergizes with IL-1β to induce ELF3 in human chondrocytes. The synergism is critically modulated by dexamethasone, by leptin receptor signalling cascade, and is dependent on NF-κB. Overall, this work highlights a novel and fundamental role for leptin in promoting and perpetuating inflammatory response in chondrocytes

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NOTCH/RBPJ/HES1 SIGNAL IN CHONDROCYTES MODULATES OSTEOARTHRITIS DEVELOPMENT

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Purpose: The Notch signaling pathway, a potent modulator of cell differentiation in many organs, is regulated by several molecules: the Notch receptors, the transcriptional effector Rbpj, and the target transcription factor Hes/Hey family members. Here we have examined the roles of these molecules in chondrocytes during the endochondral ossification process which is essential for skeletal growth and osteoarthritis (OA) development.
Methods: In vitro expression patterns were examined by real-time RT-PCR and Western blotting in cultures of mouse chondrogenic ATDC5 cells with the differentiation medium (insulin, transferrin, and sodium selenite) and primary chondrocytes isolated from the ribs of mouse embryos. In vivo expression patterns were examined by immunohistochemistry of the limb cartilage of mouse embryos and knee OA joint cartilage of the mouse experimental model. Tissue-specific knockout mice were generated by mating Sox9-Cre knock-in mice or Tamoxifen (TM)-inducible Col2a1-Cre transgenic mice (Col2a1-CreTM), with mice homozygous for a floxed Rbpj allele (Rbpjfl/fl) or those homozygous for a floxed Hes1 allele (Hes1fl/fl). For analyses of OA, TM was injected into 7-week-old Col2a1-CreTM;Rbpjfl/fl mice or Col2a1-CreTM;Hes1fl/fl mice, and the OA model was created surgically by inducing instability in the knee joints one week after this injection. OA severity was quantified by the OARSI histopathology grade 8 weeks after surgery. Functional roles of the Notch-related molecules were examined using ATDC5 cells transfected with the expression vectors, and/or the siRNA vectors. Transcriptional regulation was examined by luciferase assay using ATDC5 cells transfected with the reporter construct containing a promoter fragment of the marker genes.
Results: In cultures of ATDC5 cells and primary chondrocytes, Notch1, 2, Rbpj, and Hes1 were strongly expressed in their terminal differentiation stages during Mmp13 expression, while Notch3, 4 and other Hes/Hey members were little expressed throughout the differentiation stages. In the mouse limb cartilage and in the knee OA joint cartilage, the intracellular domains (ICDs) of Notch1, 2 were localized in the nucleus of highly differentiated chondrocytes in the hypertrophic zone and in the degraded cartilage, respectively, while they remained in the cytoplasm of less differentiated chondrocytes of the proliferative zone and undegraded cartilage. Rbpj and Hes1 were also co-expressed in the nucleus of highly differentiated chondrocytes, while other Notch ICDs and Hes/Hey family members were not detected in either cartilage. Both Sox9-Cre;Rbpjfl/fl mice and Sox9-Cre;Hes1fl/fl mice died shortly in the perinatal period; however, Sox9-Cre;Rbpjfl/fl embryos exhibited